



SALIVARY AND SERUM BIOMARKERS IN ORAL LICHEN PLANUS- A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: Lichen planus is an autoimmune T cell mediated mucocutaneous disorder, the etiology of which is still uncertain even after years of research. Numerous studies have been conducted using various markers in lichen planus in both serum and saliva, yet we don't have a reliable marker for their diagnosis. In the past decade, researchers have shown to be more biased towards studies in salivary markers or studies to compare levels of known and established serum markers with that in saliva in an attempt to prove these non invasive alternative of saliva can also be a striking alternative in diagnosing many lesions.

Objective: The aim of our study was to conduct a systematic review to evaluate and compare the serum and salivary biomarkers in lichen planus to see if serum or salivary markers are more reliable in its diagnosis.

Materials and methods: Articles published till 2019 January were chosen for the study. Articles were chosen for the study in accordance with the inclusion and exclusion criteria and PRISMA checklist was followed for selection of the articles for this study.

Results: Total of 159 articles were obtained after searching databases like PUBMED, EMBASE, LILACS and after screening the collected articles following the inclusion and exclusion criteria 14 articles were selected and analysed.

Conclusion: It was inferred that the existing knowledge is not sufficient to come to conclusion on the efficiency of markers for oral lichen planus. Also, levels of INF- gamma, TNF- alpha and TNF receptor-2 were reduced following steroid therapy suggestive of their use in monitoring effectiveness of therapy. Future studies on markers correlating to the autoimmune nature of the lesion should be done for more reliable results.

KEYWORDS : Biomarkers, Serum makers, Salivary markers, Oral Lichen planus, Interleukins, Autoimmune, Diagnosis

INTRODUCTION

Autoimmune disorders are a group of disorders wherein the body fails to recognise its own cells and produce antibodies against them resulting in tissue damage. It comprises of around 69 group of disorders. Lichen planus is an autoimmune T cell mediated mucocutaneous disorder, the etiology of which is still uncertain even after years of research.(1) Many studies have suggested a possible immunological role in its pathogenesis pointing out the role of an autoimmune mechanism where the basal of the epithelium in the skin as well as mucous membrane are targeted by the self-reactive T cells. (2)

Biomarkers are substances produced by the body whose levels are either increased or decreased in a particular disorder and thus aids in its diagnosis.(3) Though serum is the standard choice for studies in biomarkers saliva has also proven to be an attractive alternative in the diagnosis of many lesion. Saliva is in some terms an ultrafiltrate of serum and contains all components present in serum. (4) In the past decade, researchers have shown to be more biased towards studies in salivary markers or studies to compare levels of known and established serum markers with that in saliva in an attempt to prove these non invasive alternative of saliva can also be a striking alternative in diagnosing many lesions. Numerous studies have been conducted using various markers in lichen planus in both serum and saliva, yet we don't have a reliable marker for their diagnosis.(5) Also, it is important to point out the lacunae in this as the studies are mainly done focussing only on the probable inflammatory origin of the lesion and not the autoimmune reaction taking place.(6) The aim of our study was to conduct a systematic review to evaluate and compare the serum and salivary biomarkers in lichen planus to see if serum or salivary markers are more reliable in its diagnosis.

MATERIALS AND METHODS

We have performed a systematic review by considering oral lichen planus as dependent variable and serum markers and salivary markers as independent variable.

Search strategy

Systematic review involves critical analysis of multiple existing studies done in the field of interest. To answer our question of whether salivary markers were as efficient as serum markers in diagnosis oral lichen planus, a systematic literature search using a combination of keywords was done. PUBMED, LILACS, EMBASE were the search

engines that were used for collection of articles. Keywords used were biomarkers, serum, saliva, oral lichen planus in various combinations. Only studies conducted in humans were included in the study. (Figure1)

Inclusion criteria

Articles published till January 2019 were selected for the study
Only studies in humans were included in the study.
Articles with full text and published in English language were selected.

Exclusion criteria

Articles published in languages other than English were excluded.
Articles without full text were excluded.
Articles which donot mention either salivary or serum markers in oral lichen planus
Articles that correlate markers in other conditions.
Case reports, Review articles and Letter to editor were excluded.
The article was written according to the PRISMA guidelines.

To avoid bias initially two researchers were included in screening of the articles, third investigator was included when there is any dispute in agreement for inclusion or exclusion.

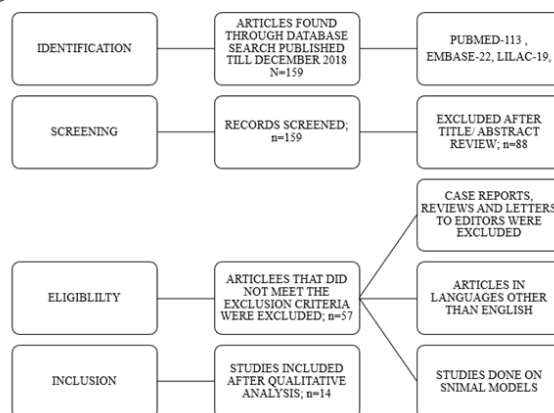


Figure1 Flow diagram representing systematic literature search on serum and salivary biomarkers in lichen planus

RESULTS

The keyword search strategy identified 159 suitable abstracts; 113 articles from PUBMED, 22 from EMBASE, 19 from LILAC and, from these 88 articles were excluded after reviewing the title and abstract after screening as they did not meet the eligibility criteria. Full text articles were obtained for 84 articles. Forty-eight articles were excluded from the study for various reasons, case reports, reviews and

letter to editor were excluded and any article published in languages other than English. Finally, after considering all the inclusion and exclusion criteria a total of 23 articles were included in the study. Figure 1 shows a PRISMA diagram for this review. The selected studies were screened, and the specific study characteristics were recorded in Table 1 and 2.

Table 1: Overview Of Studies Included In The Review- Serum Markers In Lichen Planus

AUTHOR	MARKER USED	METHOD USED IN THE STUDY	INFERENCE
Teoman Erdem, 2014(7)	P- Selectin	ELISA	Levels of P-Selectin was significantly higher in patients with lichen planus than controls. This again stresses on the inflammatory nature of the disease.
Gui-Xiang Liu 2011(6)	OPN , CECAM1	ELISA	Increased levels of OPN upregulated the levels of CECAM1 which further enhanced the activated cell survival and keratinocyte apoptosis in lichen planus.
Jing-Yu Hu 2013(8)	CCL5, IL-2, IFN gamma, MiR-125a and IL-4	ELISA	Serum CCL5 and the percentage of CCR5+CD4+ T cells elevated in OLP patients. Serum IL-2 and IFN gamma increased in OLP patients, but IL- 4 decreased. MiR-125a was down-regulated in OLP patients, and there was a negative correlation between miR-125a content and the OLP severity
Ya-Qin Tan 2017(9)	IL-21	ELISA, Flow Cytometry	Found that there is increased levels of abnormal modulation of B cell proliferation as well as increased levels of IL-21 in patients with lichen planus.

Table 2: Overview Of Studies Included In The Review- Salivary Markers In Lichen Planus

AUTHOR	MARKER USED	METHOD USED IN THE STUDY	INFERENCE
Srinivasan et al 2003(10)	CD14 and toll-like receptor-2	Western blot	Levels of sCD14 and sTLR-2 in UWS were upregulated in OLP and BMS respectively. In addition, oral epithelial cells in the saliva of patients with OLP and BMS exhibited elevated levels of CD14 mRNA and decreased levels of TLR-2 mRNA. Interestingly, presence of co-existent oral candidiasis nullified these changes
Ghallab et al 2010(11)	IFN-gamma, TNF-Alpha, and TNF Receptor-2	ELISA	Salivary IFN- γ , TNF- α , and sTNFR-2 can be detectable in ELP patients and decreased significantly after treatment with prednisone, which may reveal the possibility of using these disease-related biomarkers in diagnosis and monitoring.
Leticia et al 2011(12)	CYFRA 21-1, CA125 AND TPS	ELISA	Salivary Cyfra 21-1, CA125 and TPS are increased in OLP and might be potential biomarkers for detecting OSCC development in OLP patients
Fathi et al 2013(13)	CD4+CD25+ and MMP-9	RT-PCR	Salivary levels of CD4+CD25+ and MMP-9 were increased in patients with OLP and can be potential agents in diagnosis.
Jornet et al 2014(14)	Total anti-oxidant activity and MDA in saliva	FRAP test for Total anti-oxidant activity Spectrophotometry for MDA	Reduction in the total anti- oxidant capacity and an increase in salivary MDA in OLP patients, which could be due to the disease's exaggerated chronic inflammatory process.
Cheng et al 2014(15)	IL-8; IL-1 β ; dualspecificity phosphatase 1 (DUSP1); H3 histone family 3A (H3F3A); ornithine decarboxylase antizyme 1 (OAZ1); S100 calcium-binding protein P (S100P); and spermidine/spermine N1-acetyltransferase 1 (SAT1)	RT-PCR	Salivary OAZ1, S100P, and DUSP1mRNAs are candidate biomarkers for detecting OSCC development in OSCC patients in remission and in OLP patients
Mair et al 2016(16)	MMP-2 and -9	Gelatin zymography and Biotrak assays	MMP-2 is elevated in VE patient sera, and MMP-9 is increased in saliva of oral lichen planus patients. Therefore, these enzymes may be potential markers of disease or therapeutic targets.
Tvarijona-viciute et al 2017(17)	Trolox equivalent antioxidant capacity (TEAC); total antioxidant capacity (TAC); cupric reducing antioxidant capacity (CUPRAC); ferric reducing ability of plasma (FRAP); C-reactive protein (CRP); nitric oxide; nitrates; and nitrites	ELISA	Increased levels of nitric oxide and C-reactive protein were found in the saliva of OLP patients in comparison with BMS and control patients. Further studies to support the results are needed.
Talungchit et al 2018(18)	Salivary complement component C3c, fibrinogen fragment D, and cystatin SA	ELISA Immunoblotting for confirmation	Fibrinogen fragment D and complement component C3c exhibited increased expression in OLP patients, while cystatin SA exhibited decreased expression in OLP patients, compared with healthy control subjects. ELISA analyses indicated increased expression of fibrinogen fragment D and complement component C3c, and decreased expression of cystatin SA, in the saliva of OLP patients

DISCUSSION

In this study, we analysed 14 articles obtained through screening based on PRISMA guidelines to evaluate serum and salivary biomarkers in oral lichen planus.

Lichen planus is an idiopathic chronic inflammatory T cell mediated autoimmune disorder affecting both skin and mucous membrane.

WHO has recognised oral lichen planus as a potentially malignant oral disorder with a malignant transformation of 0.5- 1.3% , even now the exact mechanism of its malignant transformation when it occurs, is unknown.(19) various studies conducted in the past has suggested a possible role of the innate immunity in the interaction between environmental factors and the body leading o a full blown autoimmune attack mediated by the T cells on the basal keratinocytes presenting as

white plaque like lesions on the skin and mucous membrane.(20) Oral lichen planus may present as a plaque like, erosive lesion, atrophic lesion, bullous lesion etc, among this the reticular and erosive forms are the most common types with a prevalence of 2% in India.(10)

Serum biomarkers in oral lichen planus

Among the 14 studies, 4 studies were conducted using serum. Each of these studies were conducted using different biomarkers and two studies assessed were cytokines- Interleukins.(8,9) The markers assessed were all inflammatory markers and no studies have assessed any correlation with HLA antigen to demonstrate the autoimmune nature of the disease.

P-Selectin

One studied evaluated the role of P- selectin as a serum marker in oral lichen planus. It is a cell adhesion molecule seen on the surface of epithelial cells, endothelial cells and inner surfaces of blood vessels and activated platelets.(7)It is stored in granules called as Weibel-Palade bodies and in unactivated platelets its seen in alpha granules. There has been studies which proved that play an essential role in inflammation and also in recruiting of monocytes in various cardiovascular diseases.In the study conducted by Erdem et al the levels of the marker was seen to be increased in patients with oral lichen planus in comparison with control group, suggestive of its potential role in its detection.(21)

Interleukins

Interleukins are group of cytokines which are both synthesised by WBCs and act on WBCs in response to inflammatory reactions, immune responses and hematopoiesis.(22) The level of interleukins were of significance in many oral conditions like squamous cell carcinoma, oral leukoplakia, oral submucous fibrosis etc. Refer table 3 for their properties. The interleukins that were studied were IL-2,4,21. Study conducted by Jing Yu et al, showed that levels of IL-2 and interferon gamma was increased in patient with OLP in comparison to normal individuals were as IL-4 levels were lower in OLP patients in comparison with control.(8) In 2017, Qin Tan et al conducted a study comparing patients with OLP and normal individuals using levels of IL-21 in serum and concluded that levels of IL-21 was higher in OLP and had a role in the increased levels of abnormal modulation of B cell proliferation in OLP.(9)

Table 3: Properties of interleukins discussed in the study

Interleukin	Source	Target cells	Uses
IL-1	Macrophages, monocytes, B cells, dendritic cells	T helper cells	Co-stimulation
		B cells	Maturation and proliferation
		NK cells	Activation
		Macrophages, endothelium etc	Inflammation, induces acute phase reaction, fever
IL-2	T helper cells (Th1)	Activated T and B cells NK cells Macrophages Oligodendrocytes	Stimulation of growth and differentiation of T cell response. Immunotherapy of cancer and can be suppressed in transplant patients
IL-4	Th2 cells, mast cells, eosinophils	Activated B cells	Proliferation and differentiation, immunoglobulin synthesis, allergic response Proliferation
		T cells	Increased expression of VEGF
		Endothelium	Promotes adhesion of lymphocytes

IL-8 or CXCL8	Macrophages, lymphocyte, epithelial cells, endothelial cells	Neutrophils Basophils Lymphocytes	Neutrophil chemotaxis
IL-21	Activated T cells, NK cells	All lymphocytes Dendritic cells	Activation as well as proliferation of CD8+ T cells Augmentation of NK cell toxicity Proliferation of B cell

Salivary Biomarkers in Oral lichen planus

Among the 14 studies that were included in the current study, 10 were on salivary markers in oral lichen planus. Each of the studies were conducted correlated different markers, mainly interleukins, CYFA, CD etc.

Oxidative stress

Oxidative stress is caused by imbalance in the level of systemic manifestation of the level of reactive oxygen species in comparison to the ability of the body's ability to clear them off. This can cause toxic disturbances caused by the production of reactive oxygen Sil-8peices like peroxidases and free radicals damaging the body especially DNA, RNA, proteins and lipids etc. levels of oxidative stress markers are significant in neurodegenerative diseases, oral cancer, multiple sclerosis etc. In 2 studies the effect of oxidative stress was evaluated as a potential marker in oral lichen planus by assessing total anti-oxidant level and in this one study by Jorner et al, also analysed the lipid peroxidation products (MDA) also and found that the total anti-oxidant levels show a reduction with an increase in the level of MDA suggesting their potential use as a marker in oral lichen planus. (14)Tvarijonaviciute et al, analysed various factors like Trolox equivalent antioxidant capacity (TEAC); total antioxidant capacity (TAC); cupric reducing antioxidant capacity (CUPRAC); ferric reducing ability of plasma (FRAP); C reactive protein (CRP); nitric oxide; nitrates; and nitrites and found that the levels of nitric oxide and CRP were elevated.(17)

CD

CD or cluster of differentiation are markers used for the identification of cell surface molecules that act as target in immunotyping of cells. There are around 371 markers identified in human beings. They perform numerous functions in cell signalling, cell adhesion etc. There has been numerous studies that have proved the role of CD markers in oral cancer, lymphoma and various other types of neoplastic conditions, inflammatory lesions, vascular conditions etc.(10,13,23)

TLR

TLRs or toll like receptors are proteins that play an important role in innate immune system. It is expressed by white blood cells like macrophages, natural killer cells and dendritic cells. It performs function in identifying pathogenic antigens. It also plays an essential role in the mode of action of tumor associated macrophages.Srinivasan et al conducted a study comparing CD14 and Toll like receptors in OLP and BMS and found that CD 14 levels were increased and Toll like receptors were decreased in OLP and BMS.(10) Ghallab et al, conducted a study evaluating the levels of INF- gamma, TNF- alpha and TNF receptor-2 and concluded that their levels are detectable in erosive lichen planus and are reduced significantly following treatment with corticosteroids, thereby showing potential as a prognostic and therapeutic marker for LP.(11)

CYFRA 21-1

It is the soluble fragment of cytokeratin 19 which is recognised by the presence of monoclonal antibodies against it in serum. During the transformation of normal cells to malignant cells there is spontaneous caspase activity to kill the cancer cells which results in cleavage of cytokeratin 19 and causes release of CYFRA into the cancer cells. Levels of CYFRA was increased in oral squamous cell carcinoma, nasopharyngeal carcinoma etc. Leticia et al, conducted a study analysing CYFRA 21-1, CA 125 and TPS and obtained their levels through Real time PCR. These are established markers in the diagnosis of Oral squamous cell carcinoma and this study established their presence in oral lichen planus and thus may be suggestive of progression to malignancy.(24)The results obtained in our study showed that there are no effective markers in the diagnosis of oral

lichen planus. Further studies with larger samples should be done to substantiate the results of the previous studies conducted in the field and newer reliable marker should be discovered and studied.

CONCLUSION

The present study was attempted to evaluate and compare the serum and salivary markers in oral lichen planus. It was inferred that the existing knowledge is not sufficient to come to conclusion on the efficiency of markers for oral lichen planus. However, markers of oral squamous cell carcinoma like CYFRA 21-2, CA 125 and TPS gives a potential marker to evaluate malignant transformation of the lesion. Also, levels of INF- gamma, TNF- alpha and TNF receptor-2 were reduced following steroid therapy suggestive of their use in monitoring effectiveness of therapy. Future studies on markers correlating to the autoimmune nature of the lesion should be done for more reliable results.

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